COMPLEXATION: NON-CYCLODEXTRINS

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INTRODUCTION

Complexation processes, also known as complexation, are based on the ability of many well-known drugs to interact and to form new complex drugs with altered properties in comparison with a drug alone. The pharmaceutical technology and the pharmaceutical industry have long considered research and development in the area of complexation a priority. The complexation process offers new possibilities for the improvement of existing drugs (side effects, therapeutical activity, and solubility). Such drug complexes with optimized characteristics can be prepared by complexation as a result of various interactions as drug–metal ion, drug–drug, drug–excipient(s), etc.

Today, two of the greatest advances in pharmaceutical technology can be found in the field of complexation processes. These are chelatotherapy and biotechnology. The significance of chelatotherapy is evident in relation to recent increased problems connected with the pollution of the environment.

Biotechnology in relation to the pharmaceutical industry ensures new special drugs, some of which include proteins, antibodies, and peptides. Others include insulin, interferons, growth factors (GFs), and sensitive diagnostics for diseases such as hepatitis, AIDS, and herpes.

It is interesting to note that the history of medical treatment with metal ions and their compounds has been known for thousands of years. In 2500 B.C, the Chinese used gold (Aurum, Au) for medical treatment, whereas in the middle of this past millennium, the gold's compounds were considered an effective treatment against leprosy.

COORDINATION THEORY AND SOME RECENT THEORETICAL CONSIDERATIONS

The coordination theory was promoted by the Swiss chemist Alfred Werner (1866–1919) in 1891. He became a professor in Zurich in 1893, and in 1913, he received the Nobel Prize in Chemistry for his investigations of complex compounds (1). Werner found many nonorganic compounds with asymmetrical molecules that were also optically active in solutions. Such complex compounds include Co, Cr, and Fe.

The development of the chemistry of complex compounds was also promoted by the Scandinavian scientists K. Blomstrand and S. Jorgensen (1837–1914).

Gilbert Newton Lewis (1875–1946), University of California, contributed to the development of the electronic theory of the valence. He also carried out investigations on absorption spectra of organic compounds.

The atom of the complex-constitutor is the so-called central atom or central ion. In order to emphasize the difference between the central ion of the same metal in a free state, the central ion is marked with the symbol of the chemical element followed by its valency in roman numerals in brackets. Molecules and ions directly bound with the central atom are called coordinated groups (Cl, NH_3, H_2O, etc.) or intraspherical substituents (ligands or addenda) (2–4).

Ligands are "hard" or "soft" (5). The former are electronegative with electrostatic interactions, low delocalization of electron density, and formation of covalent bonds with cations. They include F^-ions and H_2O molecules. "Soft" ligands are polarizable, covalent bonds, such as chloride, bromide, iodide, sulfur-containing ligands, imidazole, etc. This division into "hard" and "soft" ligands is conventional. It is more correct to consider the series of ligands in their increasing hardness:

$$I^- < Br^- < Cl^- << F^- << H_2O,$$

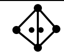
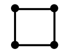
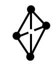
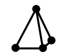
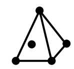
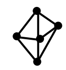
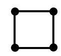
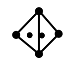
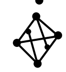
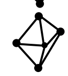
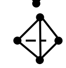
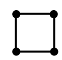
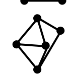
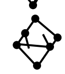
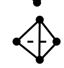
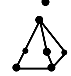
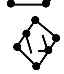
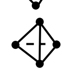
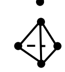
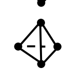
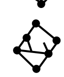
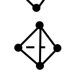
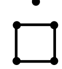
$$RS^- < R_2S << NH_3 < H_2O,$$

$$CN^- < R\text{–}NH_2$$

$$N < Cl^- < CO_2^-, \text{OH (alcohol)}$$

It should be noted that complexing ions are characterized with definite steric structures. The most widespread drug complexes with coordination number 6 are built according to the type of the octahedron, with the

Table 1 Drug configurations

Metal ion	Coordination number	Polyhedron type	Schematic presentation
Cu(I)	4	tetrahedron	
	(2)	(chain)	
	(3)	(triangle)	
Cu(II)	4	square	
	6	distorted octahedron	
	(4)	(distorted tetrahedron)	
	(5)	(square-pyramid or trigonal-bipyramid)	 
Ni(II)	4	square	
	4	tetrahedron	
	6	octahedron	
	(5)	(trigonal-bipyramid)	
Co(II)	4	tetrahedron	
	(4)	(square)	
	(5)	(trigonal-bipyramid)	
Co(III)	6	octahedron	
	(4)	(tetrahedron)	
	(5)	(square-pyramid)	
Fe(II)	6	octahedron	
	(4)	(tetrahedron)	
Fe(III)	6	octahedron	
	(4)	(tetrahedron)	
Mn(II)	6	octahedron	
	(4)	(tetrahedron)	
	(4)	(square)	

The parentheses used in the second column mark the addition of nonordinary states.

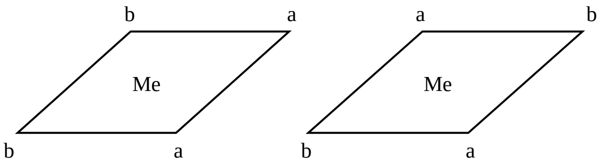


Fig. 1 Steric structures of *cis*- and *trans*-isomers.

coordinated groups oriented to the octahedron's peaks (Table 1). The drug complexes with coordination number 4 also can be built on the type of tetrahedron or on the type of plane (Table 1).

The steric structures presented explain the early detection of isomery (isomerism) of the complex compounds of Co, Pt, etc., as well as predict the number of geometric(al) isomers of the complex ion. For example, complex ions Mea2b2 may exist as two geometric isomers (*cis*- and *trans*-). The content's complication of the complex ions increases the number of geometric isomers (Fig. 1).

Consideration of the octahedral model in accordance with symmetry knowledge also has been used to predict the presence of the mirror isomerism in complex compounds with definite content and structure. Its discovery was made by Werner in 1911, and is a confirmation of the coordination theory (2, 3).

The first steric ideas were based on the pure chemical ways. The number of the isomers in the substitution reactions was determined by their comparison. These models were confirmed by X-ray diffraction analysis.

COMPLEXES FORMED BY INTERACTIONS WITH METAL IONS

Azathioprine, an immunosuppressant and cytostatic drug, forms complexes with various metal ions (6). The complexes have been investigated by the potentiometric titration method, infrared (IR) spectroscopy, etc. Azathioprine is found to form 2:1 complexes with Co(II), Cu(II), and Ni(II). The order of their stability is established as Cu(II) > Ni(II) > Co(II). Azathioprine–metal complexes have been proven by their IR spectra. The evidence of the effect of complexation was established by the absence of an absorption band at 3191 cm^{-1} (N–H stretching characteristic of a purine function in Azathioprine); e.g., the aminogroup is involved in the formation of the metal complex. The anticancer action of purine derivatives was discovered in 1949. This cytostatic drug biotransforms to 6-mercaptopurine in the body (6).

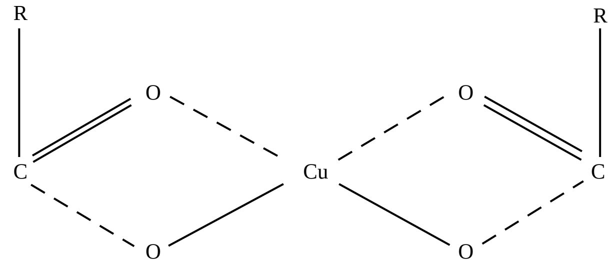


Fig. 2 The complex formation between Cu(II) ions and carboxylic groups in penicillins.

Drug interactions often constitute major problems in drug therapy. This occurs when metallic therapeutic agents and drugs (able to form complexes) are administered simultaneously (7). The metallic compounds used in pharmaceutical preparations and included in pharmacopoeias are as follows: magnesium trisilicate, aluminum hydroxide, ferrous sulfate, calcium carbonate, sodium bicarbonate, and potassium citrate. Norfloxacin, a 4-Quinolone carboxylic acid derivative with antimicrobial activity, has shown significant interaction with compounds containing Fe^{2+}, Mg^{2+}, and Al^{3+} which have led to less active complexes (unabsorbable and/or antibacterially inactive). Such complex formation is proposed as a factor responsible for the alteration of drug activity by simultaneous co-administration with metallic medicinal agents (7).

Platinum(II) complexes have antitumor activity, and have been tested against P-388 leukemia (derivatives of the substituted *o*-phenilenediamine). Their antitumor activity has been connected with many factors. These include the formation of chelate rings and their strength, the nature and the influence of different substituting groups, and the relative stability of the Pt(II) complexes (8).

Copper(II) complexes of penicillins (benzylpenicillin, phenoxymethylenepenicillin, ampicillin, amoxicillin, and carbanicillin) have been prepared. They have shown stoichiometries of the type CuL^*, CuL^*_2, and CuL^*_3 (where L^* penicilloic acids). Structures of coordination penicillin compounds have been suggested by IR spectroscopy as monoatomic bidentate ligands coordinating the Cu(II) ion through the carboxylic groups (Fig. 2).

Copper(II) ions have promoted the hydrolysis of penicillins to corresponding penicilloic acids owing to β-lactam group. The same process has been confirmed between Cu(II) ions and carboxylic groups of cephazolin, which also belongs to β-lactam antibiotics (9).

Advances include radioprotective drugs applied during radiotherapy of neoplastic diseases (10). Effects similar to

the enzyme superoxide dismutase have been found in copper complexes of Schiff's bases (derived from different amino acids and salicylaldehyde). Activity of complexes is dependent on their structures. The structural changes of their chelate rings are responsible for their effects on free radicals produced in organisms during radiation. Complexes have square pyramidal pentacoordination, which is similar to the coordination polyhedron in the active center of the Cu-dependent superoxide dismutase. The complexes used as radioprotective drugs play an important role in radiotherapy. They protect healthy tissues and cells from injurious radiation (10).

The first natural product in which the presence of the metal cobalt (Co) was shown is Vitamin B_{12} (cobalaminum). It was isolated from the liver simultaneously by K. Fokers (United States) and Lester Smith (England). The Vitamin B_{12} structure was established by Todd in 1955. The center of the molecule contains the metal Co(III) with a coordination number 6. It is connected with four mutually bound pyrol cycles. Vitamin B_{12} is used in the treatment of pernicious anemia.

COMPLEXES FORMED BY INTERACTIONS WITH EXCIPIENTS

Naltrexone (11) is a potent narcotic antagonist approximately 30–40 times more active than nalorphine, and 2–3 times more active than naloxone. Polymeric complexes as a result of hydrogen bond interactions between Naltrexone and Eudragit® have been studied. High performance liquid chromatography (HPLC), ultraviolet (UV) spectrophotometry, scanning electron microscopy (SEM), ^{1}H-and ^{13}C-nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), and hot stage microscopy (HSM) have been used to characterize naltrexone polymeric complexes. A very high efficiency in the dissolution process, as well as a significant reduction in the drug release rate from the complex, has been observed (11).

Eudragit® L (12) is a polymer with anionic character that is based on the methacrylic acid and its methyl ester (in ratio 1:1 approximately; m.w = 135000). Eudragit® L is widely used in drug preparations. A proposed complexation process allows the obtainment of Eudragit® L-Carteolol complexes. The nature of the latter was established through the use of spectroscopic techniques (IR, ^{1}H, and ^{13}C-NMR). The complex was characterized as intermolecular association (polymer–drug), and the drug interacted with ammonium (maximum carteolol salt content in complex—22%). Comparative studies of carteolol and morphine complexes have shown differences. These differences are explained by the different chemical structures related to the amino group of the two drugs (12).

The excipients ethylcellulose (13) and pectin (14) show the possibilities for complex interactions of the type intermolecular H-bonds. The latter result in increased therapeutic activity of the amoxicillin trihydrate (granules) and nystatin (plaque). The nature and complex character of these interactions have been investigated by means of IR spectroscopy and X-ray diffraction (13, 14).

Indomethacin was introduced into medical practice in 1963. Its anti-inflammatory activity is much greater than aminophenazon and hydrocortisone, and it is a much stronger antipyretic than aminophenazon. The properties of indomethacin in complex formations have been studied. The drug interacts with various agents, such as zinc (15), calcium glycerophosphate (CGP) (16), polycarbophil (17), chitosans (18), 2-(*N*,*N*-dimethylamino) propionate (19), and epirizole (20). The indomethacin complexes show better characteristics when compared to the drug alone. Improvements in water solubility, increased dissolution and absorption rates, and increased bioavailability have been proven, as well as decreased side effects (gastrointestinal ulceration and hemorrhage). Some of the zinc–indomethacin complex characteristics are as follows: m.p.—232°C (decomposition), IR spectrum—1586 cm^{-1} (asymmetrical stretching of carboxylate anion), ligand and metal ratio 2:1, and two molecules of crystal water (15).

The preparation methods of Indomethacin complexes and their advantages have been described in detail. These include: 1) Zn–indomethacin (15)—economical and less time consuming; 2) indomethacin–CGP—simplicity, facility of design, and nearly cost-free production (of the complex) on a large scale (industrially (16); 3) the concentration of polycarbophil can be used as a key step in suppositories (17); and 4) indomethacin–epirizole complex (20)—new spherical crystallization technique applied without further processing as granulation.

Propranolol–methacrylic acid copolymer complex (21) has been evaluated as a potential prolonged releasing drug. Propranolol content is found to be 68% in the complex. The complexation process has been defined as positive interaction of a high degree between propranolol and the polymer (specific ion–ion interactions and hydrophobic binding to the overall complexation process). The slow release of propranolol from the complex may be due to hydrogel formation when the drug (the tablet) is exposed to the dissolution medium. The complex has been investigated by differential thermal analysis (DTA) and IR- and UV-spectroscopic methods.

Chitosan is a cationic polymer used for controlled drug delivery. It forms polyion complexes (interpolymer) as a result of its interactions with anionic polymers. The polyion complexes and their basic properties have been investigated for their pharmaceutical application (22). The specific properties of the complexes (chitosan–sodium alginate and chitosan–sodium acrylate) are due mainly to rigidity or flexibility of the polymer chains. The former is stable to pH change, and the latter is quite sensitive to pH change, which makes them applicable to the design of more precisely controlled drug delivery systems. Fourier transform infrared spectroscopy (FTIR), elementary analysis, and viscosity measurements have been used to explain the nature of these complexes (22).

Recently, the tranquilizer action of phenothiazine derivatives has been connected with the flexibility of their molecules (23). They form complexes with charge transition. These complexes have been obtained as a result of the interactions between phenothiazine derivatives, dextrans, and pectins. IR spectroscopy, X-ray diffraction, UV spectroscopy, and Dreiding models (a 3-D research model) have been applied (24). Hypochromic effects (changes in the band's intensity) in UV spectra have been observed. The degree of complex binding correlates with the concentrations of dextrans.

COMPLEXES FORMED BY DRUG–DRUG INTERACTIONS

A new complex of Ind and epirozole (molecular ratio equal to 2:1) has been prepared by the spherical crystallization technique and proven by means of IR spectroscopy and X-ray diffraction (20).

ANALYSIS

Drug complexes require application of numerous methods of analysis (physico-chemical, biological, etc.) for their complete characterization (25). The choice and combination of these methods are connected mainly with their effectiveness in establishing the type of the complexes as well as proving their mechanism. Today, the commercial availability and economical convenience (26, 27) of the drug complexes (their preparation, analysis, and manufacturing) propose a new strategy and balance for pharmaceutical technology (the pharmaceutical industry, respectively). In this context, the combination of IR spectroscopy and X-ray diffraction are methods of choice (28).

Complexes obtained by interactions between aliphatic amines and carboxylic acids (23) have a structure type of the ion pair and complex composition 1:1. Their IR spectra do not have the characteristic absorption band for free νC=O in the region from 1780 to 1700 cm^{-1}. However, new characteristic absorption bands appear for the carboxylate anion in 1680–1560 cm^{-1} (ν_{as} COO^-) and 1400–1300 cm^{-1} (ν_s COO^-). Some additive bands also appear: The vibration NH^+HO^- (in the region 2800–2200 cm^{-1}), as in the salts in solid state, and the bands δNH_2^+ or δNH_3^+ at 1620–1600 cm^{-1}, and δCO_2 at 670 cm^{-1} (where ν is stretching or valence vibration and δ is deformation vibration). The complex with composition 2:1 can be formed as a result from the addition of a second acid molecule to an ion pair (in increased amine concentration). Characteristic absorption band of the carboxylate anion shifts to the lower values of the wave numbers (as compared to the complex with 1:1 composition) (23).

Electron Paramagnetic Resonance

Electron paramagnetic resonance (EPR) gives basic information on the complexes containing Cu and Fe ions (in drugs or proteins) mainly for the character of their bonds and ligands. Free organic radicals also give EPR signals, which makes interpretation of spectra very complicated (29).

Electron Spectra (UV–Visible Spectra)

Usually, the intensive bands appear in UV–visible regions that are connected with the charge transition (between ligand and metal ion or between ligand atoms). The band positions (in charge transition) are dependent on the nature of metal and ligand as well as on their relative ability to oxidation and restoration) (23).

Nuclear Magnetic Resonance (NMR)^{1}H

These spectra are applicable in conformation analysis, e.g., stereochemistry. They are used to provide very necessary information on the role of metal and on the nature of metal complexes.

Complexometry

This is a quantitative (titrimetric) analysis for drugs containing bismuth (Bi), calcium (Ca), magnesium (Mg), plumbum (Pb), and zinc (Zn), among others. The titration is performed with solutions of polyaminocarboxylic acids and their salts, the so-called complexons (I, II, and III or Trilon B, respectively). They are able to form stable,

water-soluble complexes in stoichiometry (1:1) with the drug metals. The titration is carried out in the presence of one of the metal-indicators used (eriochrome black T, murexide, or xylenolorange). The requirements for the metal indicators include their reversible interactions with the metals of drugs analyzed, unstable complex formation, and color differences in free or complex states (2). Therefore, complexometry is an analysis in principle based on the complex formation ability of some drugs.

CHELATOTHERAPY: COMPLEXATION PROCESSES IN THE HUMAN ORGANISM

The treatment of poisoning with metals includes the use of drug chelating agents. The latter form stable and nontoxic complexes with metals and are quickly eliminated from the body.

Criteria of the Drug Choice as Chelating Agents

Chelatotherapy requires comparative evaluation of the chelating drugs. The following must be taken into consideration when choosing chelating drug agents for a given metal: 1) stable binding with the metal so that it may be concurrent to the biological ligands in the organism; 2) selectivity; and 3) nontoxicity.

The selectivity of the drug ligand to a definite metal can be evaluated by analysis of the formation constant. In cases where the selectivity is decreased or absent, unwanted side effects have been observed as a result of the complexation with other metals (such as calcium and zinc). The chelating drug agents attack heavy metals. In the organism, the metal will be in a lipophilic medium. When the ligand is lipophilic, the lipophilic complex cannot have toxic action. If the ligand is adequately lipophilic, it can penetrate the membrane of the depo metal forming a lipophilic complex (29).

A discussion of some chelating drugs follows.

Dimercaprol (BAL): This drug preparation appeared during the World War I and was used for treatment of battle poisoning along with luisit ($ClHC{=}CHAsCl_2$). The luisit action is based on the binding with —SH groups.

BAL as a drug ligand makes stable bonds with arsen (As) and, thus, is able to remove it from the luisit molecules. BAL also has been used in the treatment of poisoning with other metals (Hg, Ca, Au, Ti, Tl, and Bi). BAL forms solid (strong) bonds with a series of metals; consequently, it must be used in low concentrations. The use of BAL requires more attention since the organic compounds of Hg increase their concentrations in the brain in the early stages of the poisoning (30).

EDTA (30): In this drug preparation, calcium is bound as complex and can be displaced from the ions of heavy metals. These metals are bound loosely in the biochemical systems of the tissues and are liberated easily from them.

Water-soluble stable complexes with low toxicity are formed with EDTA and are very rapidly eliminated with urea. EDTA is given by injection in acute and chronic poisoning with Pb, Cd, Co, Hg, U, It, and Ce.

Penicillamine: Penicillamine is used in the treatment of cronic copper accumulation (Wilson's disease).

X-ray diffraction investigations explain the structure of this complex. The central ion is a halogen surrounded with eight Cu(I) atoms with sulfur donors. The coordination sphere of the Cu(II) is completed with amino groups of the penicillamine (29).

Anticancer drugs: Anticancer drugs and their metal complexes appear as *cis*- and *trans*-isomers. The *cis*-isomers of the dichlordiaminoplatin(II) (*cis*-DDP) and the tetrachlordiaminoplatin(IV) are known as the most effective complexes. The former *cis*-isomer of platinum(Pt;II) has a large application in the treatment of cancer of the ovaries and testicles. *Trans*-complexes have shown toxicity and do not possess non-anticancer action.

All of the anticancer drugs (*cis*-DDP) have side effects, such as nausea, diarrhea, decrease of the hemoglobin levels, and destruction of the kidneys. The latter is connected with the difficulty of creating and supporting the high liquid content in patient organisms during treatment.

Thus, it is extremely important to determine the most effective methods that allow in vivo low drug concentrations. Some basic protocols have been established on restoration of in vivo complex Pt(IV), disintegration polymeric platinum compounds, and slow evacuation of blocking inert ligands. Other platinum complexes also have been examined (29).

The method of circular dichroism has been applied in investigations that have determined the reactions between Pt(II) compound and nucleosides, nucleotides, and DNA. Many antimicrobial preparations are excellent ligands. The activity of some of the antimicrobial preparations has been based on their complexing with metal ions. For example, increased effectiveness of tetracycline has been observed after its coordination with Ca^{2+}. The complex is more lipophilic and very oil-soluble, which explains its transportation through the cell membrane. The opposite situation occurs when the ion-metal is toxic and the coordinated antibiotic serves as carrier through the membrane.

Diuretic drug preparations have promoted urine formation. They are derivatives of mercury propanol $RCH_2CH(OH)CH_2H_gX$, where R is a polar hydrophilic group. The mercury diuretic preparations act as ferment inhibitors (latter containing—. They also inhibit adenosine triphosphate (ATP). These properties led to the use of mercury drug compounds in the treatment of bacterial infections. In these cases, they interacted with —SH groups of the bacteria proteins.

Interactions between Drug Preparations and Metal Ions in Humans

Many drug preparations can interact with metal ions. Therefore, they function in humans as chelating agents and form complexes. In some cases the therapeutic activity of a drug compound can be explained by inhibition through a chelated metal ion. For example, disufiram (tetraethylthiouram disulfide) is used in the treatment of chronic alcoholism. It inhibits aldehyde oxidase, and the ethanol metabolism is interrupted in the stage of formation of the acetaldehyde. The latter leads to an unpleasant feeling. It makes the alcohol (ethanol) nontransportable in its later use. The complex with metal ion can lead to formation of the neutral oil-soluble particles able to penetrate the cell membranes. The metal ion acts as a carrier of the drug substance preparation (29).

Drug preparations able to interact with metal ions include acetazolamide, amphetamine, aspirin, ethambutol, phenacetin, nialamide, disulfiram, and thioacetazone. The evaluation of new drugs includes the emphasis of the following important requirements: 1) the drug's strength and the ways (or mechanisms) of its action; 2) the side effects of the drug and its metabolites; 3) its stability in vivo; and 4) its possible interactions with other drugs.

Consideration of these requirements is extremely important. The possible interactions between two or more drugs, taken simultaneously, make their use dangerous or change their therapeutic activity (either increase or decrease). Only the nonionized drug compounds are oil-soluble. Sometimes, drug preparations do not appear active since some of their metabolites are in pharmacologic active forms. Drug characteristics are connected with the whole drug molecule or with receptors. The degree of ionization of the drug in vivo has been determined by the values of pKa and pH of the organism liquids. The effectiveness of the sulfonilamides against infections is stimulated by the presence of the NH_2 groups. The latter prevents the growth and the multiplication of bacteria. The applications of the metal complexes against numerous microorganisms and against strains highly resistant to the traditional antibiotics have great importance (29). The form and the distribution of the charges in the complex give information concerning the necessary pharmacological properties.

Metals as Drugs

Lithium (Li) has been used in the treatment of bipolar disorder (manic depression) for approximately 50 years. Over 2000 British patients use Li for this disease. The illness is characterized by alternative states of depression and mania, or overexcitement. At times, the cycle of mood changes continue for several weeks or for one year with good results (intervals) between the phases. In Texas, a correlation has been noted between the influence of Li-salts and the drinking water as well as the increase in visits to psychiatric (mental) public health hospitals.

The compounds of gold were first applied in the treatment of rheumatoid arthritis in 1927, and are used even today in the treatment of severe cases.

Thiomalate (salt of ester of the malic acid) also has been used very successfully to treat severe cases of rheumatoid arthritis. A complete cure or a significant improvement has been noted in approximately 50% of patients using this treatment modality, with 40% of patients exhibiting side effects. Thiomalate is also toxic and possesses cumulative action. Minimal concentrations of the gold compounds reach many cells and remain in the body for years (29).

Complexing and Nonmetals as Microelements

Many nonmetals are important microelements necessary for the normal function of organisms. Some polyvitamin preparations contain microelements as microadding. Their therapeutic and toxic action depends on the concentration. Therefore, the presence of these microelements in the organism in proper amounts is extremely important. Examples of nonmetals include boron (B), silicon (Si), selenium (Se), arsenic (As), chlorine (Cl), fluorine (F), and iodine (I).

Studies on the influence of boron have shown that its compounds as borate have inhibited some fermentative reactions. In addition, the influence of boron results in membrane destruction (29).

The lack of silicon (Si) leads to destruction of bone structure and connective tissues (29).

Deficiency of selenium (Se) influences the concentration of vitamin E. The latter is an antioxidant and protects membranes from oxidation. The ability of Se to protect organisms from the poisoning with Hg or Cd are well known. The normal concentration of this microelement is 0.09–0.2 mkg/cm^3 (29). Interestingly, the

concentration of Se in the blood of New Zealanders shows a decrease (0.068 mkg/cm^3).

Fluorine (F) and its metabolites are of importance in protecting teeth from caries. Fluorine is included in calcium hydroxyapatite, and it promotes the precipitation of calcium phosphate $Ca(PO_3)_2$ and accelerates the remineralization. The necessary concentration of Fluorine added to drinking water to prevent caries is approximately 1 mg/L. Application of higher Fluorine concentrations (above 8 mg/L) leads to fluorosis. This is a disease that is characterized by a disturbance in the function of the thyroid gland. A long-term application of fluorine leads to intensive mineralization (possible precipitation of calcium sulfate), deformation of bones with possible accretion, and calcification of the connections (29).

Iodine (I) has a great role in the function of the thyroid gland. It takes part in a complex biochemical synthesis schema and interacts with hormones. Deficiency of iodine is characterized weakness, feelings of cold and dryness, and yellow-colored skin. The treatment of these symptoms is achieved with iodine or thyroid hormones (29).

BIOTECHNOLOGY

Drugs as special products of biotechnology have an important role in the pharmaceutical industry. Biotechnology is based on the progress of genetic engineering and fermentation technologies. These advances make possible a host of new products, some of which include peptides, proteins, and antibodies, and are used as therapeutics or diagnostics.

Potential drugs, such as interferons, interleukins (as well as other lymphokines), growth factors, and plasminogen activators are also being used as diagnostics. They are very sensitive to many diseases, such as hepatitis, herpes, and AIDS (31).

The beginning of biotechnology stems from 300 small biotechnology companies (often with roots from academic laboratories) that operated in the late 1970s. Today, the list of large American biotechnology companies is long. In addition, the governments of the United Kingdom, France, Germany, and Japan have launched major efforts to assist their countries' development of biotechnology. Some of the large European biotechnology companies include Novo Industri in Denmark and Celltech in the United Kingdom, but the majority of biotechnology companies are in the United States (approximately 75%).

The properties of insulin (5) have been investigated in many ways—as a drug, a protein, and a hormone. Its complex chemical structure has been proven (two polypeptide chains bound by means of two disulfide bridges). Insulin appears as monomers, dimers, tetramers, or hexamers, depending on the number of its associated molecules. Thus, insulin monomers form dimers, which aggregate in hexamers in the presence of zinc (Zn) in neutral pH. Insulin in concentrations 10^{-5} M (molar) exists as dimers. Many of the latter have been formed as a result of van Der Waal's interactions. Insulin molecules are also bound with hydrophobic forces and hydrogen bonds. The complex chemical structure of insulin explains its ability to take part in complexation processes. Heavy atomic reagents, together with insulin, form various complexes (e.g., with cations of Lantanides, lead [Pb], thallium [Tl], and uranium [divalent group: UO_2]. The latter possesses a linear geometry $(O{=}U{=}O)^{2+}$. Insulin complexes have been formed with different mercury (Hg) reactants. T.B. Blundell is well known as the British scientist who deciphered the structure of insulin. (He is also known for his work on the building of proteins.) Crystallography of insulin and its complexes have been studied by means of X-ray diffraction and electron microscopy.

The presence of Calcium (Ca^{2+}) is necessary for blood coagulation and subsequently prevents hemorrhaging from tissue injuries. The mechanism consists of cascade-type process in which stages are connected with the presence of Ca^{2+}. Many of the so-called factors of blood coagulation are well known. Vitamin K is necessary in the biosynthesis of the factors IX, X, and VII and of prothrombin. The 1,25-dioxiform of vitamin D_3 facilitates the process of Ca^{2+} reception from the intestines. Accumulation of Ca^{2+} and its release from humans is a complex system, and also includes vitamin D.

Almost all proteins (ferments; redox-, transport- and spare-proteins; hormones; and antibodies) have been studied by X-ray diffraction analysis (5).

Ferments are catalysts. They change the rapidity of the biochemical reactions without displacing the equilibrium ratio.

Redox-proteins are connected with cell organelle as mitochondria, chloroplasts, membranes with specific functions, and high oxidation–reduction potential.

Transport- and spare-proteins (the hemoglobin with its four subunits) ensures the rapid transport of oxygen to a definite place. They have been studied by X-ray diffraction analysis since 1937.

Hormones regulate intracellular metabolic processes. Insulin intensifies transfer (transport) of the glucose in the cell and decreases its level in the circulating blood. Glucagon increases the sugar level in the blood. The two hormones are synthesized by the pancreas. Antibodies discern foreign bodies in an organism.

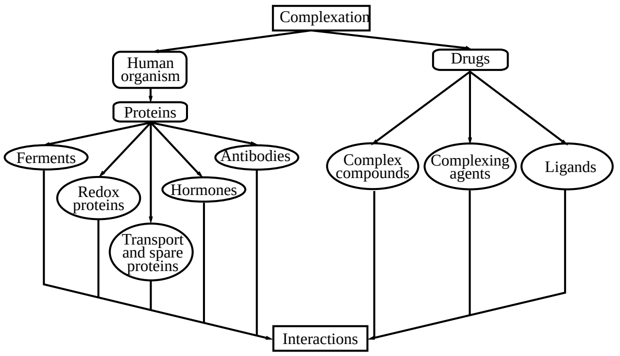


Fig. 3 Schematic presentation of the complexation processes—drugs and human organism interactions.

DISCUSSION

The preparation of drug complex compounds and the use of their qualitative properties have enormous importance in the pharmaceutical industry. Complex compounds have also taken part in the processes of the vital activity of humans as inner-complex compounds in hemoglobin, tissues, etc. (Fig. 3). Complex bound metals are important components of the ferments (in particular, the oxidative ferments).

A systematic view on the topic of "complexation" and complexes in the literature shows a great number of important research results. This expanding field covers studies of various systems, including antibody–antigen complexes, proteinase–inhibitor complexes (32), lipid-based delivery vehicles, metal complexes with DNA base pair, and aqua ligand in the coordination sphere of metal (33). The metal–modified structures are dominated by these metal-base interactions. However, the structural role of the water molecules in the complexes is quite apparent, as suggested by crystallographic studies (33).

A possibility for the next generation of therapeutics is connected with the complexation processes of proteins with virus membranes, which destabilizes them (34). This knowledge is of interest for future use in genetic medicines. An area of much promise is complexes of polycations with DNA. They result in major improvements. Cationic liposome-based gene delivery accounts for 9–12% of ongoing gene therapy clinical trials in the United States and Europe (35).

Today, many computer programs assist in the investigation of new drug ligand designs, geometry of interactions, and conformational flexibility of the potential ligands (26).

CONCLUSION

Hughes (29) is quite right when he concludes "It is evident today that the metal ions are controlling (are checking) the various biological processes and that life is based on the organic and inorganic chemistry." Finally, modern pharmaceutical technology has successfully applied the advanced knowledge of many sciences (chemistry, biochemistry, molecular biology, pharmacology, etc.) to solve the various complex problems of drug manufacturing (the pharmaceutical industry) and of drug development. These scientific approaches, to integrate and use all the best of human thought for human health, confirm the pharmaceutical industry as an important interdisciplinary science today and in the future.

Recent research on drug complexation can be described as a tunnel that is being dug from two opposite ends with the efforts of many scientists. On one side are those who solve the problems with drug complexation, while on the other side are those who solve them with protein complexation. When they meet in the middle of the tunnel, the mechanisms of the interactions between drugs and human organisms will be elucidated.

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